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AN EVALUATION OF THE ENVIRONMENTAL FATE AND BEHAVIOR OF MUNITIONS MATERIEL (TETRYL AND POLAR METABOLITES OF TNT) IN SOIL AND PLANT SYSTEMS

Preliminary Evaluation of TNT-Polar Metabolites in Plants

Project Order No. 88PP8853

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A preliminary study was conducted to elucidate the nature of the polar metabolites reported for plant tissues and xylem exudates following root accumulation of trinitrotoluene. Studies focused on the nature of the proposed conjugates of TNT-residues in xylem exudates of bush bean plants. Use of enzymatic methods failed to demonstrate that the conjugates were carbohydrate based, but acid hydrolysis indicated that the conjugates may be protein based. Of the five polar conjugates isolated from exudates, the presence of aminodinitrotoluene isomers and one unknown TNT residue was demonstrated.

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EXECUTIVE SUMMARY

Trinitrotoluene (TNT), unlike hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), undergoes rapid conversion or metabolism to polar forms following accumulation by terrestrial plants. This results in some uncertainty as to the potential food chain risk that may be imposed by TNT when it is released to the environment. A prior study at the same laboratory suggested the presence of potential environmentally important polar TNT and TNT-metabolite conjugates following root accumulation of trinitrotoluene by plants. To begin to address this question, a preliminary study was conducted to clarify and elucidate the nature of these polar metabolites present in plant tissues and xylem exudates.

The study focused on the nature of the proposed conjugates of TNT-residues contained in xylem exudates of bush bean plants, which represent the transport form(s) for TNT residues between roots and shoot tissues. Use of enzymatic methods failed to demonstrate that the conjugates were carbohydrate based. However, acid hydrolysis of TNT-residue-0.containing components isolated from xylem fluids indicated that the conjugates may be protein based. Of the five polar conjugates isolated from exudates, the presence of aminodinitrotoluene isomers and one unknown TNT residue (more polar than the aminodinitrotoluene isomers) was demonstrated. Determining the importance of these findings awaits further elucidation of the role of protein and possibly DNA adduction.

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1.0 INTRODUCTION

Munitions materiels currently used as propellants or as explosive charges include trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and 2,4,6-trinitrophenylmethylnitramine (tetryl). These materiels and their decomposition or combustion products may enter the environment as a result of both production and manufacturing activities, and field usage and disposal (Small and Rosenblatt 1974; Kitchens et al. 1978; Spanggord et al. 1983; Ryon et al. 1984).

The presence of these specific munitions-related components in the environment is not, however, indicative of the existence or the severity of an environmental impact. Persistence, and therefore accumulation of the contaminant, can be further influenced by physical processes such as its relative chemical stability and/or the presence of biologically mediated degradative processes.

These physical processes may be dependent on the sorptive processes of the soil and the contaminant's relative water solubility. Organic contaminants, such as the munitions materiel, may undergo chemical partitioning as they enter terrestrial and aquatic environments, thus affecting their short-term accumulation and mobility.

Biotic processes have been clearly indicated as being effective in the degradation and detoxification of a variety of organic xenobiotic compound classes, and they may operate in a similar fashion with munitions. With this in mind, biotic processes important for assessing the relative long-term behavior of munitions-related materiel released to the environment have been the subject of prior reports. Important factors include 1) the extent to which soil microbes can degrade and/or modify the contaminant; 2) the extent to which the parent compounds and their major decomposition products are accumulated by food-chain plants; and 3) the extent to which plant-accumulated contaminants are metabolized and degraded. The third of these is the focus of this preliminary study to elucidate the chemical fate of TNT residues once they are accumulated by terrestrial plants.

1.1 REVIEW OF RELATED LITERATURE

1.1.1 Toxicity of TNT

There are several recorded instances of human toxicity associated with TNT exposure. Documented cases over the last 60 years report instances of liver damage and anemia among chronically exposed munitions workers (Sax and Lewis 1989). In the environment, TNT and its derivatives have been reported to inhibit growth of freshwater algae at 2 to 15 µg/g (Smock et al. 1976) and to negatively affect other green algae and oyster larvae; to inhibit the growth and metabolism of microorganisms generally (Klausmeier et al. 1973; Nay et al. 1974) as well as, among the vertebrates, fish (Nay et al. 1974), rats and mice (Lee et al. 1975). Furthermore, TNT was found to be toxic to lower plants (duckweed) at levels in excess of 1 µg/g (Schott and Worthley 1974). With the exception of studies by Smock et al. (1976) and Schott and Worthley (1974), no chemical analyses were performed on the materials accumulated by the organisms. However, little if any information is available as to the the chemical fate of metabolized TNT residues in higher plants, let alone the subsequent toxicity of these metabolites to other trophic levels along the food chain.

1.1.2 Chemistry and Analytic Methods

Palazzo and Leggett (1986) were the first to address the analysis of explosive residues in plant tissues. Since their initial report, methods described by Cataldo and co-workers (Cataldo et al. 1989, 1990; Harvey et al. 1990, 1991) represent significant advances in the analytical methodology for determining explosives residues in plant tissues. Of particular interest is the use of methodologies that indicate a rapid conversion of TNT and related residues to more polar, although unidentified, chemical forms.

1.1.3 Plant Uptake and Metabolism

The literature contains few studies related to higher plant soil/plant fate and bioavailability of TNT. One such study was performed using hydroponically grown yellow nutsedge to assess the uptake, toxicity, and metabolic transformations of TNT (Palazzo and Leggett 1986). This study showed shoot and particularly root growth to be inhibited at 5 µg/g.

Although this point was not noted by Palazzo and Leggett, these toxicity symptoms are characteristic of dinitroaniline herbicide damage, which might be expected based on the chemical structures of TNT and the aminodinitrotoluenes. Chemical analysis showed that >90% of all tissue-extractable material was present as 2- and 4-ADNT, with only a small amount of TNT being recovered. Because these species were not observed in the nutrient solutions, it is assumed that they were metabolic detoxification products.

Detailed plant fate studies related to TNT have been conducted recently (Cataldo et al. 1989; Harvey et al. 1990). In bush beans grown in hydroponic solutions and exposed to ^{14}C-TNT, TNT-derived ^{14}C was found to be localized primarily in the roots, with only a small amount (<9% of the total radiolabel in the plant) having been transported to the shoot and leaves even after 7 days of exposure. When the leaf tissue from these plants, which contained approximately 8.6% of the total label in the plant, was homogenized with water and subsequently extracted with diethyl ether, only 4% of the label present in this tissue (less than 0.3% of total plant label) was found to partition into the ether layer; 57% of the leaf's label (less than 4.9% of total plant label) remained in the water layer, and the remaining 40% (3.4% of total plant label) was retained in the pellet. This indicated that the TNT that had been transported to the leaf from the root was metabolized to a very polar, and perhaps conjugated, form in this tissue.

In an attempt to free possible conjugates from the tissue and identify the water-extractable components, Cataldo et al. (1989) implemented acid hydrolysis immediately after homogenization of the tissue. When a subsample of the leaf tissue was hydrolyzed with 1 N HCl for 1 h prior to extraction with diethyl ether, 23% of the leaf tissue radiolabel (1.9% of total plant label) was ether-extractable, with 41% (3.5% of total plant label) remaining in the HCl layer. This experiment illustrated that approximately 19% of the TNT or TNT-derived compounds (~1.6% of total plant label) in the leaves of these plants had been metabolized to HCl hydrolyzable conjugates after only 7 days. Interestingly, acid hydrolysis did not increase the amount of radiolabel that was solubilized from the pellet. In both the acid hydrolysis and the water experimental conditions, slightly over 60% of the total radiolabel in the leaf tissue (>5% of total plant label) was consistently found to be solubilized in the aqueous or ether layers after 7 days of exposure.

When the stem and roots of the bush bean were analyzed in a similar manner, it was observed that almost twice as much of the total label in the roots was contained in the ether-extractable fractions (TNT and aminodinitrotoluene isomers) as in the HCl and water fractions (Cataldo et al. 1989). Stem tissue contained concentrations of TNT and the aminodinitrotoluene isomers that were intermediate between the large concentrations in the roots and the scant concentrations in the leaves. Furthermore, the proportion of the total label remaining in the pellet following extraction for these two tissues was consistently lower, at 28±10%. This distribution indicates progressive formation of polar compounds as TNT metabolites were transported from the root to the leaves.

In an attempt to identify the primary transport forms of TNT, additional experiments were conducted to examine the xylem exudate of bush beans grown in hydroponic solutions containing ^{14}C-TNT (Cataldo et al. 1989). Analysis of stem exudate has several inherent advantages over the analysis of stem tissue. Paramount among these is the fact that stem exudate contains only xylem transport products, or those metabolites being transported from root to shoot tissues, whereas stem tissue contains both transport products and storage forms. Additional advantages include the non-viscosity of the exudates, the absence of pigments, and the relatively simple chemical composition of exudates.

In that study, the roots of an intact 27-day-old bush bean were submerged in a 25-ppm TNT solution containing 10 µCi of radiolabeled TNT. The stem was severed and 1.3 mL of exudate collected over a period of 5 h. This sample was divided into two aliquots. The first of these were lyophilized and then reconstituted with 100 µl of 50:50 MeOH:H_2O. A radiochromatogram of this sample is presented in Figure 1.1. The overwhelming majority of radiolabel eluted in the dead volume of the reverse-phase column, indicating the highly polar nature of the transport products. A very small amount of radiolabeled compound did not elute until after a retention time of 13 min. This corresponds to the retention time of the aminodinitrotoluene isomers.

The second 0.65-mL aliquot of exudate was acid-hydrolyzed with HCl at 100 °C for 1 h prior to extraction with diethyl ether. The aqueous layer was made basic by the addition of

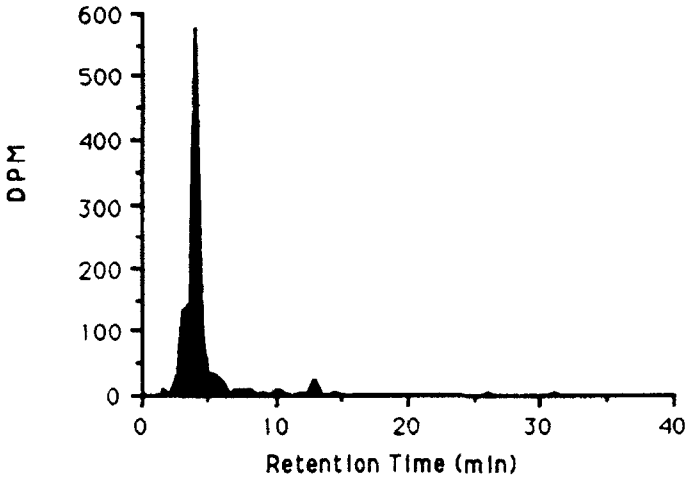

Figure 1.1. Radiochromatogram of Bush Bean Exudate (Cataldo et al. 1989)

NH_4OH and again extracted with diethyl ether. The radiochromatogram obtained on the acid-hydrolyzed material (Figure 1.2) represented a surprisingly simple chromatographic profile that contained only two major constituents. The highest peak (retention time of 13 min) was due to the aminodinitrotoluene isomers; the second-highest peak (retention time of 22 min) was due to a compound of unknown composition. This experiment clearly demonstrated that TNT is transported from the root to leaf tissue almost exclusively as conjugates of the aminodinitrotoluene isomers. Unfortunately, this information was not pursued during the duration of this contract and the characterization of the conjugates could not be completed.

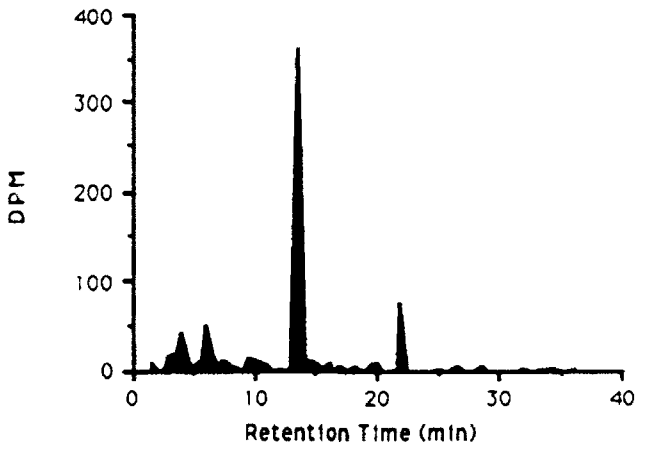

Figure 1.2. Radiochromatogram of Acid-Hydrolyzed Bush Bean Exudate (Cataldo et al. 1989)

1.2 STUDY OBJECTIVES

The objectives of the study described in this report were to assess the chemical composition of the TNT-conjugated polar transport moiety (or moieties) in bush beans. Particular emphasis was placed on elucidating 1) the composition of the transport conjugate (carbohydrate or protein) and 2) the enumeration of the number of possible TNT-derived transport forms within the xylem of the plant.

1.3 TECHNICAL APPROACH

Ongoing research with TNT (Cataldo et al. 1989), RDX (Cataldo et al. 1990), and other organic xenobiotics for U.S. Army Medical Research and Development Command has clearly shown the need to understand the critical aspects controlling the behavior and chemical fate of environmental contaminants. This study continues to address the environmental fate and behavior of TNT in plants. The methods and approaches previously developed for TNT are being applied to understanding the composition of the principal TNT-metabolites that may have significant environmental effects.

2.0 MATERIALS AND METHODS

2.1 PURITY AND ANALYSIS OF TNT SOURCES

2.1.1 Chemicals and Standards

A series of standards, including 2,4,6-trinitrotoluene, 2-amino-4,6-dinitrotoluene, and 4-hydroxylamino-2,6-dinitrotoluene, were obtained from Chem Service (West Chester, Pennsylvania), the U.S. Army THAMA SARM Repository, and Oak Ridge National Laboratory, respectively. Purity was >98% for each compound.

2.1.2 Chemical Analysis of ^{14}C-TNT by HPLC

Uniformly ring-labeled ^{14}C-TNT was obtained from New England Nuclear (E.I. du Pont de Nemours & Co., Boston, Massachusetts). The purity of the TNT radiolabel to be used for these experiments was determined by radiochromatography. The radioiochromatogram, presented in Figure 2.1, indicates a purity of 99.26 %. This purity was sufficient for our metabolic studies, as shown in the example of a standard concentration curve (Figure 2.2), and the radiolabel was used without further purification.

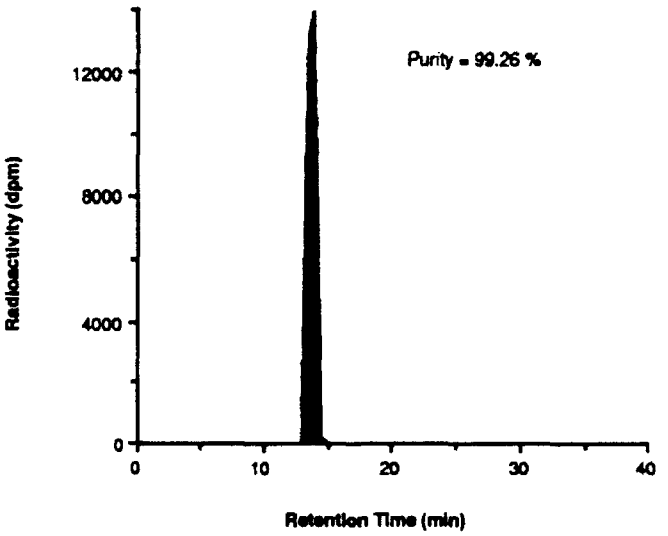

Figure 2.1. Radiochromatogram of ^{14}C-TNT Illustrating the High Degree of Purity

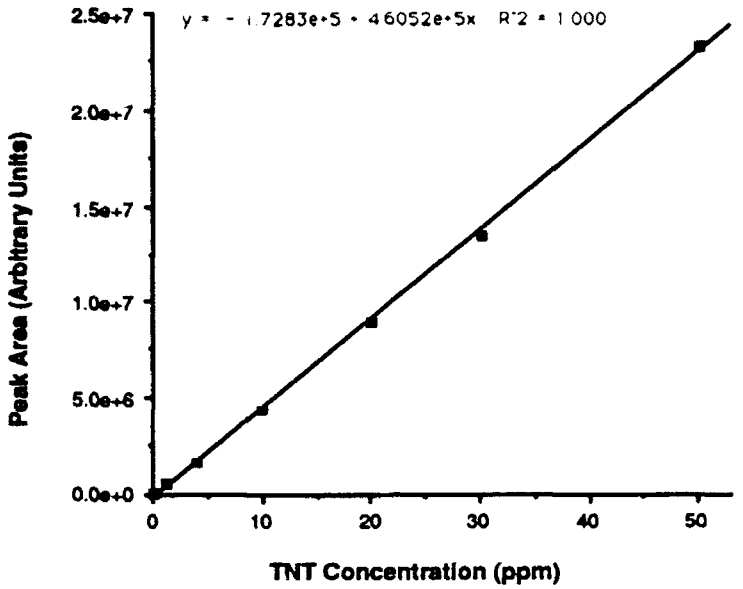

Figure 2.2. Representative Standard Curve for TNT

2.2 PLANT CULTIVATION AND SAMPLING

2.2.1 Plant Selection and Cultivation

The chemical fate of TNT-polar metabolites in plants was evaluated using bush beans (*Phaseolus vulgaris*, cv. Blue Lake Bush). All plants used were grown from seed. Plants were grown for 18 to 26 days in 600-mL beakers containing 500 mL of hydroponic nutrient solutions described previously (Garland et al. 1981). These contained 150 mg KCl, 120 mg $MgSO_4$, 946 mg $Ca(NO_3)_2 \cdot 4\ H_2O$, 68 mg KH_2PO_4, 0.06 mg $ZnSO_4 \cdot 7\ H_2O$, 0.69 mg H_3BO_3, 0.017 mg $CuCl_2 \cdot 2\ H_2O$, 0.024 mg $Na_2\ MoO_4 \cdot 2\ H_2O$, 0.022 mg $MnCl_2 \cdot 4\ H_2O$, and 0.60 mg Fe^{+3} (as ethylenediaminetetraacetic acid ferric sodium salt) per liter. The solution pH was adjusted to 5.8 and brought to volume daily with fresh solution. Plants were maintained in a controlled-environment chamber with a 16/8-h light/dark cycle (~450 $\mu E/m^2$ per sec, phototsynthetically active radiation, at leaf surface or 850 W/m^2), a day/night temperature cycle of 28/22°C, and 50% RH. All solutions were bubbled with filtered air to provide aeration and mixing as described by Cataldo et al. (1978). The beakers were jacketed in opaque sheaths to protect the roots from light, and to minimize the photolysis of TNT.

2.2.2 Xylem Exudate Collection

In experiments aimed at elucidating the transportation forms of TNT, bush bean exudate was collected from plants grown in hydroponic solutions containing ^{14}C-TNT, using the method described by Fellows et al. (1989). For each collection, ten 29-day-old bush bean plants were placed overnight (14 h) in 500-mL beakers containing 400 mL of a TNT-amended nutrient solution. Each 400 mL of nutrient solution contained 20 µCi of radiolabeled TNT at a final concentration of 25 µg/g. The following morning, the plants were taken from the growth chambers within the first 30 min of the start of the light cycle (the time of highest exudation rate) and the stem was severed with a razor blade approximately 6 to 8 cm above the solution level. The first 100 µL of stem exudate was blotted away and the severed stem fitted with a latex tube connected to Teflon tubing that led to a scintillation vial. The vial was maintained at 4 °C during exudate collection (2 to 4 h). The exudates from all of the plants were pooled, yielding 4 to 5 mL of exudate having an activity of ~10 dpm/µL, and analyzed as described below.

2.3 CHEMICAL FRACTIONATION AND ANALYSES

2.3.1 Enzymatic Digestion for Conjugates

The enzymes β-glucuronidase, β-glucosidase, protease, papain, and ficin were obtained from Sigma Chemical (St. Louis, Missouri). The buffers used for the incubations are listed in Table 2.1. Enzymes were incubated with bush bean exudates at 37 °C for 24 h; the mixtures were subsequently extracted with diethyl ether and the organic and aqueous phases separated. The diethyl ether layer was evaporated to dryness and the residue reconstituted with methanol. Volumes of the methanol solution and the aqueous layer were determined gravimetrically prior to removal of 100 µl of each solution for liquid scintillation counting.

Table 2.1. Buffer Compositions Used for the Enzymatic Hydrolysis

Enzyme Treatment	Buffer Used for Hydrolysis	Units Added (as defined by Sigma[a])
β-glucuronidase	0.008 M phosphate buffer at pH = 6.8	200
β-glucosidase	0.5 M acetate buffer at pH = 5.0	110
Protease	50 mM phosphate buffer at pH = 7.5	35
Papain	0.05 M sodium acetate pH 4.5	554
Ficin	2.0 M NaCl and 0.03 M cysteine pH = 5.0	41

[a] Sigma Chemical, St. Louis, Missouri.

2.3.2 Residue Analysis

The high-performance liquid chromatrography (HPLC) system consisted of a Waters Model 600E pump and system controller. The methanol extract (20 µL) of soil or plant tissue extracts was injected by a Waters WISP 710 automatic injector onto a Beckman Ultrasphere 5-µm octadecyl silica column and the components were separated by a linear solvent program at a flow rate of 1.0 mL/min. The solvent system was water/acetonitrile, with a 20-min gradient from 40% to 100% acetonitrile. Components were detected by ultraviolet (UV) absorption at 254 nm (Waters Model 490E detector), with a detector sensitivity of 0.008 AUFS. Peak areas obtained from a Hewlett-Packard 3390 integrator were used for quantitative measurements.

Radiochromatographic detection was extensively used for unambiguous identification of transformation products derived from TNT. During selected chromatographic runs, the column eluate was collected in 0.5-mL increments for a total of 30 min. Each fraction was assayed for radioactivity by liquid scintillation spectrometry. Radiochromatograms were generated by plotting the disintegrations per min (dpm) in each successive aliquot as a function of retention time. Transformation products and/or metabolites identified in this manner were collected by repetitive HPLC runs to accumulate enough material for subsequent mass-spectral studies.

2.3.4 Radioanalyses

Samples for radiocarbon analysis were oxidized by total combustion in a Packard Model 306 oxidizer (Packard Instrument Co., Downers Grove, Illinois) to determine the amount of radiocarbon associated with each sample. Combusted samples and liquid samples were counted using a Beckman 9800 Liquid Scintillation Spectrometer (Beckman Instruments, Palo Alto, California) with appropriate quench correction.

3.0 RESULTS AND DISCUSSION

Results of xylem exudate studies with bush bean (Cataldo et al. 1989; Harvey et al. 1990) indicated that TNT is transported as polar conjugates to aerial portions of the plant, where it undergoes further metabolic alteration. Subsequent polar TNT metabolites are sequestered within the plant and are not appreciably transpired as volatile organics or $^{14}CO_2$. Chemical fractionation of plant tissues grown in TNT-containing hydroponic solution indicated that less than 12% of the incorporated radiolabel was due to TNT, 2-amino-4,6-dinitrotoluene, or 4-amino-2,6-dinitrotoluene. Most of the radiolabel was found in either a polar ether-extractable fraction or a more polar non-ether-extractable fraction. The preponderance of these previously unknown, polar ether-extractable TNT transformation products was a major finding of the previous studies. The objective of the present effort was to further elucidate the chemical nature of the polar residues.

3.1 CHARACTERIZATION OF PLANT METABOLITES OF TNT

In view of the chemical complexity of TNT polar metabolites in plants, exploratory experiments were initiated to optimize the chances of identifying potential classes of conjugates. These experiments involved analysis and characterization of polar xylem transport forms.

3.1.1 Enzymatic Hydrolysis of Xylem Exudate

The xylem exudate of hydroponically grown bush beans exposed to ^{14}C-TNT was subjected to enzymatic hydrolysis followed by solvent extraction and HPLC analysis of the organic phase for the aminodinitrotoluene isomers. Several enzymes were used to clarify the nature of the conjugates; these included β-glucuronidase, β–glucosidase, protease, papain, and ficin.

If a particular enzyme is active in cleaving polar conjugates from the TNT metabolites, the resulting non-polar radiolabel would be expected to partition into the diethyl ether layer on extraction. Results of the enzymatic experiments are provided in Table 3.1. In general, the enzymes failed to effectively cleave the conjugates recovered from xylem exudates. In the

Table 3.1. Percentage of Diethyl Ether-Extractable Radiolabel After Enzymatic Hydrolysis of Bush Bean Exudate Collected from a Plant Exposed Hydroponically to TNT-Amended Hydroponic Solution

Enzyme Treatment	Aqueous Layer	Diethyl Ether Layer	Mass Balance
β-glucuronidase	72.9	12.9	85.8
β-glucosidase #1	47.2	31.8	79.0
β-glucosidase #2	55.9	27.4	83.3
Protease	76.6	8.6	85.2
Papain	65.2	22.9	88.1
Ficin	66.1	17.8	83.9
0.5 M Acetate Buffer (pH = 5.0)	60.3	30.4	90.7

duplicate analyses performed with the β-glucosidase, 31.8 and 27.4% of the radiolabel was ether-extractable after hydrolysis. Incubation of bush bean exudate in acetate buffer that did not contain enzyme gave 30.4% ether-extractable radiolabel. It is likely that this is due to the formation of extractable ion pairs formed with the acetate buffer. As can be seen from Table 3.1, enzyme treatments resulted in very little partitioning of the radiolabel into the diethyl ether layer. These results indicate that cleavage of the polar TNT conjugates did not occur to an appreciable degree with the enzymes investigated. Thus the conjugates are likely neither carbohydrate nor protein based.

3.1.2 Radiochromatographic Studies of Bush Bean Exudate

Further investigation focused on radiochromatographic examination of the bush bean stem exudate. For this experiment, the exudate used came from bush bean plants exposed to high-specific-activity hydroponic solutions. Hydroponic solutions contained 25 µg/g TNT and a total of 100 µCi ^{14}C-TNT per 500-ml beaker. Plants were placed in these solutions overnight

and exudate collections made 12 h later. Exudate used for these experiments was collected during the first 2 h following decapitation.

To characterize the polar conjugates contained within the bush bean stem exudate, gradient reversed-phase chromatography was employed with a weaker solvent system than was previously used (Cataldo et al. 1989). Conditions commonly employed by our laboratory for profiling TNT and the aminodinitrotoluene isomers start with a mobile phase composition of 40% acetonitrile. To profile the polar TNT-conjugates, the initial mobile phase composition was changed to 100% water. The gradient ramp was from pure water to 50% acetonitrile over 25 min. At this point, the gradient was continued at a faster rate to 100% acetonitrile in an additional 15 min. Direct injection of the bush bean exudate under these conditions resulted in the chromatogram shown in Figure 3.1. A radiochromatogram corresponding to the UV trace is shown in Figure 3.2. The principal peaks that contain radioactivity are numbered 1 through 5 in both these figures. This experiment clearly demonstrated that TNT is transported from the roots to shoots as a number of discrete polar metabolites.

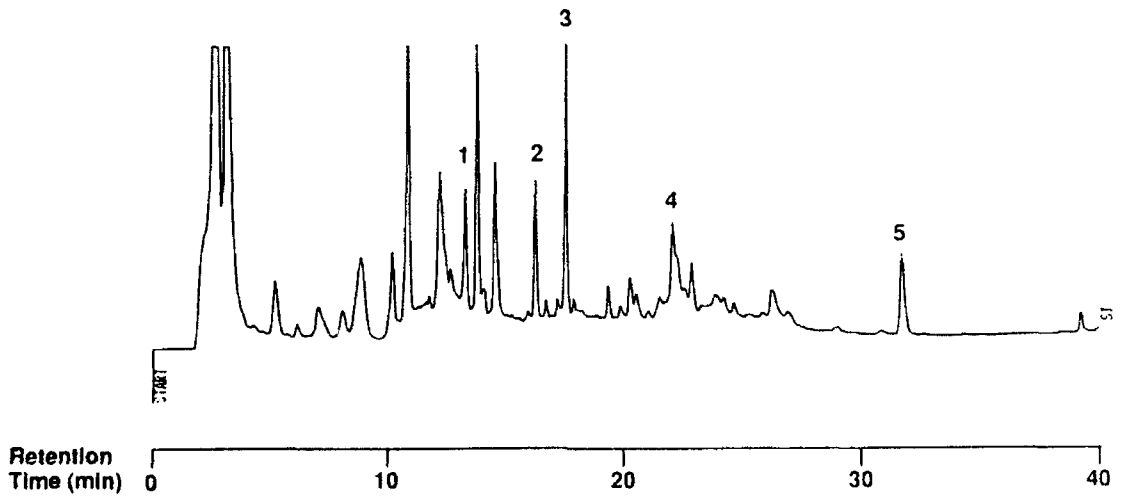

Figure 3.1. Representative HPLC Chromatogram with UV detection (254 nm) of Stem Exudate from a Bush Bean Plant Maintained in TNT-Amended Hydroponic Culture

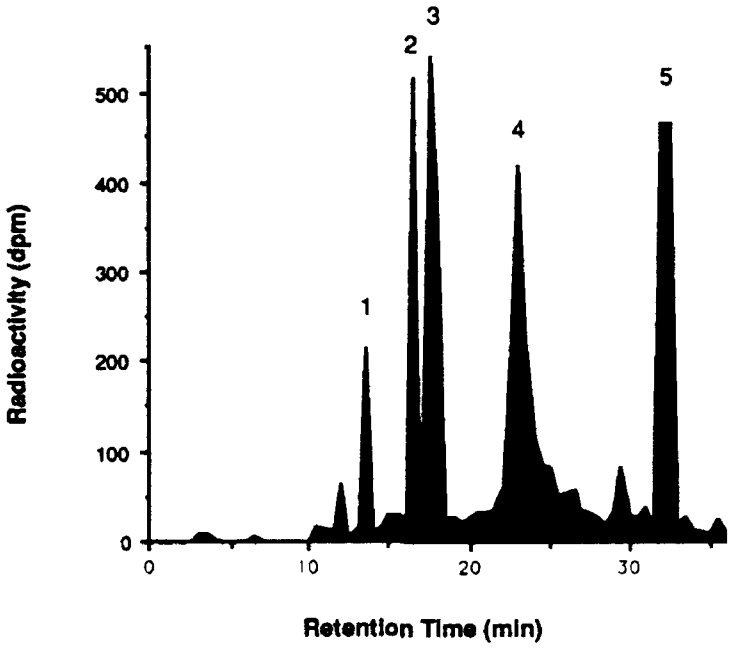

Figure 3.2. Radiochromatogram of Bush Bean Stem Exudate from a Plant Maintained in a *TNT-Amended Hydroponic Culture*

As previous experiments demonstrated (Cataldo et al. 1989), acid hydrolysis of the polar conjugates results in the formation of aminodinitrotoluene isomers and an unknown TNT metabolite that elutes with a retention time of 22 min. Next, the column eluate was pooled for each individual peak shown in Figures 3.1 and 3.2. The acetonitrile solvent was then removed by a gentle stream of nitrogen. Acid hydrolysis was accomplished by addition of 5.0 mL of 1 M HCl followed by heating at 100 °C for 1 h. After cooling, the hydrolysis mixture was extracted with 5.0 ml of diethyl ether. The aqueous layer was made basic and again extracted with diethyl ether. The ether layers were pooled and evaporated to dryness, and the residue was dissolved in methanol. A 20-µL aliquot of this solution was counted by liquid scintillation spectrometry; the remainder of the solution was analyzed by radiochromatography.

Radiochromatograms of the acid-hydrolyzed peaks are shown in Figures 3.3 through 3.6 for Peaks 2 through 5, respectively. The quantity of radiolabel collected for peak 1 was not sufficient for radiochromatographic characterization.

The radiochromatograms presented in Figures 3.3 and 3.4 show hydrolysis products from peaks 2 and 3 (Figure 3.2) and indicate the presence of aminodinitrotoluene isomers. The amount of radiolabel collected for peak 4 was only sufficient to give an indication of the components liberated by acid hydrolysis. The radiochromatogram of acid-hydrolyzed peak 4 is shown in Figure 3.5. This radiochromatogram suggests that the acid-hydrolyzed peak 4 contains both the aminodinitrotoluene isomers and an unknown TNT metabolite that is more polar than the aminodinitrotoluene isomers (retention time of 8.0 min). Finally, acid hydrolysis of peak 5 yielded the same unknown metabolite, as well as lesser amounts of the aminodinitrotoluene isomers (Figure 3.6). This unknown metabolite is not a diaminonitrotoluene isomer as indicated by the fact that injection of a 2,6-diamino-4-nitrotoluene standard had a retention time of 4.36 min when analyzed under the same chromatographic conditions.

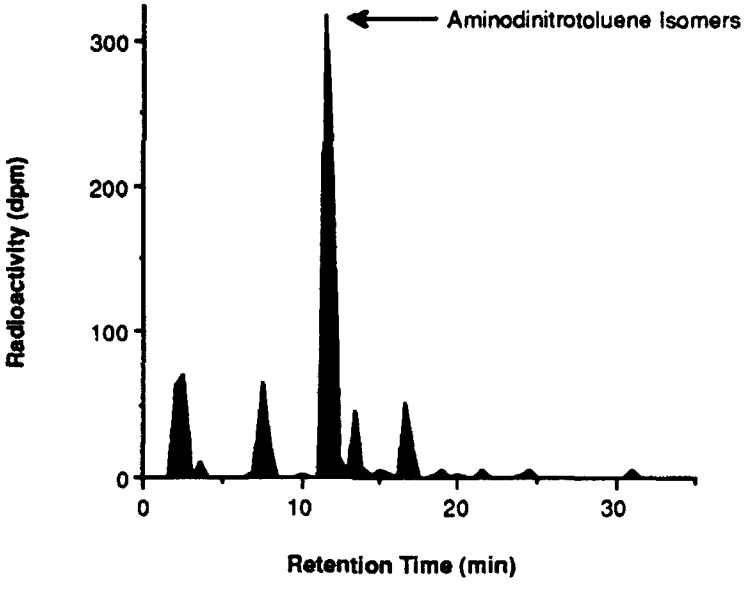

Figure 3.3. Radiochromatogram of Acid-Hydrolyzed Peak 2

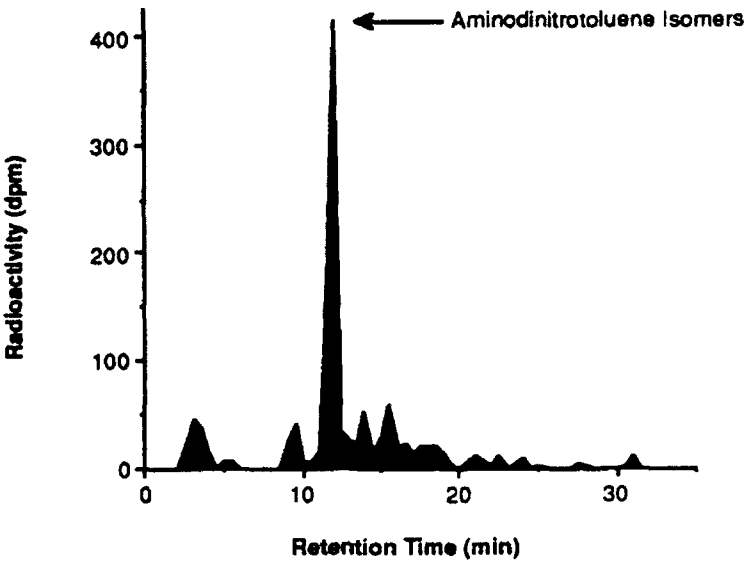

Figure 3.4. Radiochromatogram of Acid-Hydrolyzed Peak 3

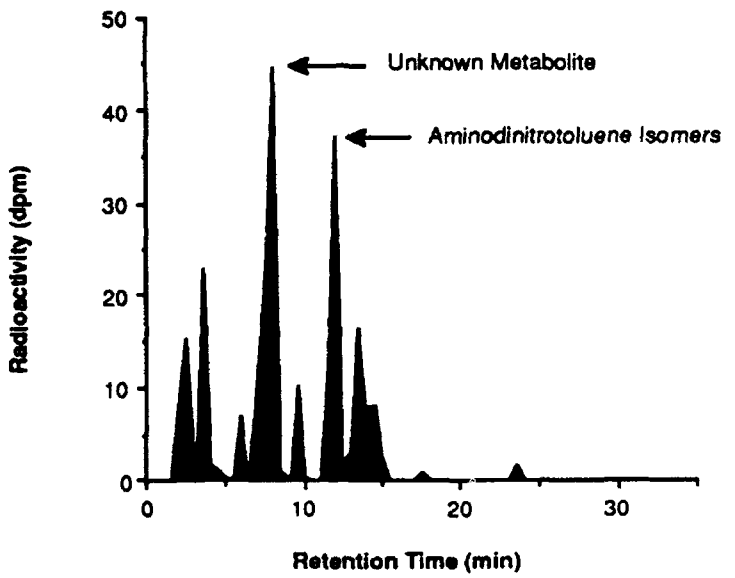

Figure 3.5. Radiochromatogram of Acid-Hydrolyzed Peak 4

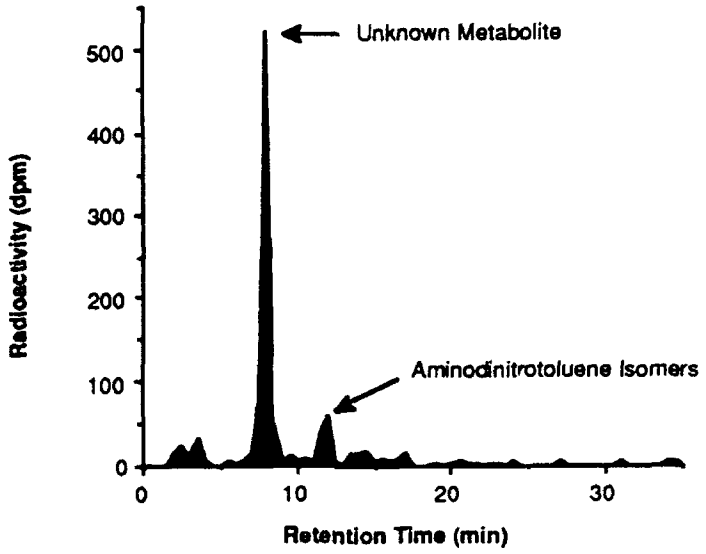

Figure 3.6. Radiochromatogram of Acid-Hydrolyzed Peak 5

A quantity of the unknown metabolite that elutes at 22 min was collected from acid-hydrolyzed bush bean exudate and has been archived for further chemical characterization.

4.0 SUMMARY AND CONCLUSIONS

The results of this preliminary study failed to elucidate the nature of the conjugates associated with the TNT-residue transport forms in xylem exudates. However, results clearly show that

1) the majority of the TNT and its residues that are accumulated by plants are polar, and not simple transformation products,

2) the transport and likely tissue storage forms are conjugated in some consistent fashion,

3) the TNT residues can be released from the supposed conjugates by acid hydrolysis to yield one known metabolite (aminodinitrotoluene isomers) and one unknown TNT metabolite (retention time of 8.0 min) that is more polar than the aminodinitrotoluene isomers, and

4) the TNT-residue conjugates are likely not simple carbohydrate or related conjugates.

The results, while not definitive, do suggest that the polar conjugates may be small peptide-based molecules, since they are readily released by acid hydrolysis. This suggests that their ultimate chemical fate and potential impacts in plants and other trophic levels of the food chain may reside in their adduction with proteins and possibly DNA. If so, their potential to adversely impact the environment and humans requires further study.

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